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Thermophilic Digestion of a Mixture of Domestic Sewage Sludge and Cellulose Materials

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Thermophilic Digestion of a Mixture of Domestic Sewage Sludge and Cellulose Materials

by

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CONTENTS

INTRODUCTION

In the low grade or mechanical purification of domestic sewage wastes, the liquid is made to pass through a series of mechanical arrangements that successively separate all coarse material. Most of the impurities in the sludge have such a small particle size that they can be removed only by means of sedimentation in special troughs. It is found that the sludge which collects on the bottom of such troughs has a high content of organic substances. The high content of faeces and household wastes in this material means that such wastes cannot be stored, for sanitary reasons, in an untreated form since they would soon start to putrefy. One way of converting the sludge into a harmless, non-smelling form is to subject it to a special treatment by means of a bacterial process. This is performed in closed tanks, and gives a digested sludge which has been freed from the greater part of the easily decomposed compounds and which is biologically stable from a practical point of view. The part of the original sludge which has been removed is mainly converted to a mixture of methane and carbon dioxide. The gases resulting from the digestion can be burnt and therefore used for heating purposes in the purification plant.

While the sludge from domestic sewage possesses, because of its highly diversified components, considerable amounts of bacterial nutrients, which of course greatly stimulate the digestion process, it can happen that the sludge from industrial sewage is completely dominated by a component that can be decomposed only with great difficulty. Thus, the sludge from pulp factories consists almost completely of cellulose fibers, and the sludge liquor is poor in nitrogen and phosphorus. It would therefore seem *a priori* attractive to try and treat the sludge from domestic sewage together with such an industrial sludge and thus to exploit the excess of nutrient matter in the former in order to facilitate the digestion of the latter. The method would of course require that the relative sizes and positions of the industry and community were suitable for such a purpose.

The object of the investigation reported here was to study, on a laboratory scale, the decomposition of sludge from domestic sewage mixed with cellulose sludge from a pulp factory. The work embraced, in the beginning, the study of purely basic problems and also, at the same time, the accumulation of practical experience concerning the apparatuses used and the methods for analysis. Eventually, the investigation became of more definite practical importance when experiments were begun which were more directly connected with actual conditions in a purification plant.

II. MATERIALS AND METHODS

A. Materials

(a) Bleached sulphite pulp

For both initial trials and also accurate fundamental experiments, we have used cellulose in the form of a thin crape paper made from bleached sulphite pulp. For experiments on thermophilic cellulose fermentation, a large quantity of this material with the following routine analysis data had been procured:

Table 1

Routine analysis data for bleached sulphite pulp in the form which was used in these experiments

α-cellulose, %	89.6
β- " "	3.7
γ- " "	6.4
total	99.7
Ash, %	0.13
Pentosans, %	2.3
Roe-number	0.1
Viscosity at 20°C., centipoises	40.5
Mn, p.p.m.	1.9
Fe, "	16.0
Cu, "	5.4
SiO_2 "	70.0

(b) Noll fiber

Several different samples of noll fiber were procured during this work. Routine analyses were performed for ash, crude fibre (see p. 7) and resinous material (1). Because of the presence of these constituents as well as of lignin, the degree of digestion for noll fiber is only 70 % of that for bleached sulphite pulp (as found in our own experiments).

Table 1 a

Routine analysis data for a sample of noll fibre

Ash, %	16.5
Crude fibre, -"-	58.5
Resinous material, -"-	5.8
	80.8
Lignin and other substances not accounted for, %	19.2

B. Equipment

(c) Fresh domestic sewage sludge

Sludge from a purification plant in Stockholm was steam autoclaved in 40 l portions for 4 hours, homogenized after cooling in a Waring blendor and stored at + 2°C.

B. Equipment

The digestions were carried out partly on a 8 l scale and partly on a 0,75 l scale. In the former case, the apparatus used (cf. fig. 2) consisted of a 10 l round-bottomed flask supplied with a glass stirrer for continuous stirring. The flask was immersed in a water thermostat equipped with double temperature regulation because of the risk for overheating. The gases formed were collected in graduated glass cylinders over a saturated NaCl solution. Test samples of the digestion liquor were collected by suction. Three such apparatuses were in continuous use. For smaller scale experiments, 1 l flasks were employed without stirrers (cf. fig. 1) and with temperature regulation and gas collection arranged for in the same way as for the larger apparatuses. The test samples were forced out by means of nitrogen. Sixteen 0.75 l digestions could be performed simultaneously.

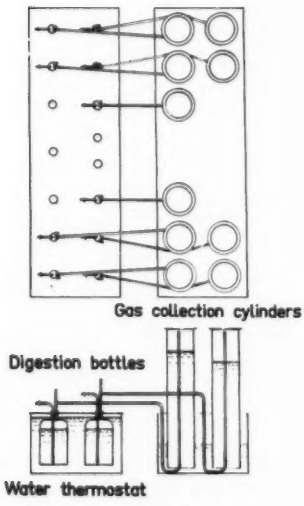

Fig. 1

Apparatus for laboratory digestions on a small scale.

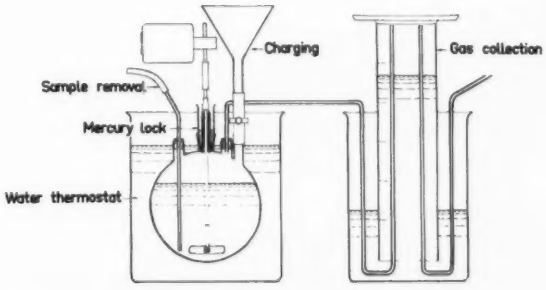

Fig. 2

Larger apparatus for laboratory digestions.

C. Standard methods used for the experiments

(a) Digestions on a 0.75 l scale

The smaller apparatuses were employed in accordance with section B above using 675 ml of a nutrient solution and 75 ml inoculation.

Nutrient solution:

NH_4Cl	1.0 g	$FeSO_4$, 7 H_2O	0.02 g
K_2HPO_4	1.0 "	$MnSO_4$, 4 H_2O	0.04 "
$MgCl_2$, 6 H_2O	0.25 "	biotin	2 µg
$CaCl_2$, 6 H_2O	0.1 "		
Difco yeast extract		2.0 g	
Sodium thioglycollate		0.4 "	
Water to 1000 ml			

In certain experiments, the biotin and yeast extract were omitted. In those experiments where the nutrient solution did not contain any solid digestion material, 20 g long-fibred asbestos was added to each flask as a "carrier" for the bacteria.

The flasks were incubated at 58.0 ± 0.2°C. The pH was held at 7.5 by adding either NaOH or HCl.

(b) Digestions on a 8 l scale

The larger apparatuses described under B above were employed using 8 l nutrient solution and 10 % inoculation. The addition of nutrient salts was different in various cases as is apparent from the description of the individual experiments. "Standard addition" means, in this case, nutrient salts in those amounts per litre which are given under (a). The flasks were incubated at 58.0 ± 0.2°C and the pH was adjusted as in (a).

D. Analysis methods

(a) Analysis of residual digestion material

Total solids and ash

In an aliquot, the solid material was separated by centrifugation, washed with water, transferred to a weighed porcelain crucible, dried overnight at 105° and then weighed. The dried sample was converted to ash using an electric oven at red heat and the residue obtained was weighed.

Cellulose as crude fiber (2)

The determination was performed on an unwashed sample because washing was shown to be of no importance in this case. The sample was treated successively with H_2SO_4 and KOH under such conditions that the hemicellulose, as well as the protein and fat were dissolved out from the cellulose itself which was thus obtained as residue.

Crude protein

A nitrogen determination was performed on a centrifuged, washed sample and the crude protein content was calculated by multiplying the nitrogen content by the factor 6.25 (3). It can be discussed whether this factor should, for the sake of correctness, be increased a little – however, since the analysis was mainly directed towards a comparison of the values obtained, no change was made in the value for this factor.

Ether soluble (2)

An unwashed sample (washing of no importance) was extracted after HCl-treatment using ethanol, ethyl ether and petrol ether – the dry substance in the extract was reported as ether soluble.

(b) Analysis on the digestion liquor

pH

The pH was determined electrometrically on a filtered sample.

Volatile acids

A 20 ml portion of the sample was steam distilled after acidification with H_3PO_4 to a pH of about 2.5 and a 10-fold distillate was collected. The amount of volatile acids was reported as g/l acetic acid.

Ammonia-N

The determination was performed on centrifuged digestion liquor by distilling off the ammonia after adding $Ba(OH)_2$ to a pH of ca. 10.

Phosphate as PO_4

A sample of the digested liquid, which had been centrifuged, filtered and evaporated to a small volume, was treated repeatedly with HCl and was then combusted in an oven according to (4). The colorimetric determination was then performed according to (5).

(c) Analysis on the digestion gas

While the CO_2 content in the gas could be easily determined by using an usual Orsat apparatus, it was found however that the determination of CH_4 and H_2 was very difficult due to the fact that the Pt catalyst in the combustion tube was poisoned by the volatile sulphur compounds in the gas. This difficulty could be overcome to a certain extent by washing the gas with a cadmium acetate or mercury acetate solution before combustion but the method remained, despite this precaution, so hazardous that we decided instead to use a Perkin-Elmer gas chromatograph. The greatest difficulty with this method was that H_2 could not be determined accurately on the SiO_2 column which was found suitable. Because the main interest in the composition of the digestion gas is directed towards how the carbon in the material is divided between CH_4 och CO_2, it was considered that the H_2 determination was of minor importance especially as the H_2 content normally only constitutes a negligible fraction of the total amount of gas.

In the 8 l digestions, the O_2 content in the gas was measured regularly by means of the Orsat apparatus in order to keep a check on any possible leakage. Since the gas in the digestion vessels was always kept under a slight underpressure, the presence of oxygen in the gas was a sensitive indication of leakage in the system. However, leakage was found to occur only very rarely and then only in such a small amount that there was no effect at all on the digestion process.

(d) Remarks concerning the accuracy of the analyses

Several of the analytical methods used in this investigation are subject to considerable systematic errors, partly due to the fact that they have been used on materials of different types to those for which they had been originally developed. This circumstance was however relatively unimportant due to the fact that the analyses were not intended for the obtaining of absolute values but rather for a comparison between results obtained at different times. Irrespective of the systematic error, it should therefore be possible to determine the relative variation in a certain component during the digestion process.

III. EXPERIMENTAL

1. Digestion Experiments

(a) Preliminary experiments

Trial digestions were made in order to test the apparatus and the analytical methods. By using only ethanol and lower fatty acids as digestion material, it was possible to separate the methane fermentation from the initial decomposition processes otherwise occurring. The optimum temperature for the thermophilic methane fermentation of ethanol was found to be 58 – 60 °C – a value which is higher than what is given in the literature as the optimum for the complete digestion of domestic sludge (6). The explanation is probably that certain of the auxiliary processes occurring in the last mentioned case have a lower temperature optimum than the methane fermentation itself.

In connection with these trial experiments, the maximum conversion ability of the 8 l apparatuses was also determined using ethanol as digestion material. It was found that the gas production, obtained on adding ethanol by means of a continuously operating charging device, could be held at 14 l/day – this corresponds to an addition of 13.5 g ethanol per day. The volume of liquid in this experiment was 7 l. A daily addition of 15 g ethanol caused overloading, the gas production quickly falling almost to zero.

Fresh domestic sludge is, in many respects, a difficult material to work with – not least because the composition varies. Trials were therefore made to replace the fresh sludge by a "synthetic" material whose composition was based on the most important components in the fresh sludge. One of these main components consists of a mixture of proteins with different origins. Trials were therefore made on the digestion of bleached sulphite cellulose mixed with protein materials of different types (fresh beef, casein, egg albumin).

It was found that the cellulose was digested easier than the protein material, a proportionally larger addition of the latter resulting in a decreased gas production. Of the protein materials investigated, egg albumin was converted easiest and the beef slowest – this was however not due to the egg albumin being soluble while the meat was added in a minced form but rather to differences in the chemical composition. The meat particles were in fact dissolved up rapidly in the digestion liquid. The tendency towards the accumulation of the lower fatty acids was how-

ever greater in the meat digestion.

The maximum conversion capacity of the 8 l apparatuses was, in these experiments, about 8 g digestion material (i.e. total solids) per day provided that this material consisted of equal parts of cellulose and egg albumin.

Eventually, however, it became clear that it would be both difficult and tedious to devise a "synthetic" digestion material with as convenient digestion properties as those of fresh domestic sludge. The mixture of cellulose and protein was converted slower and with a greater tendency towards an accumulation of volatile acids than fresh sludge. Attempts to enrich the "synthetic" digestion material by adding vitamins of the B group did not have any appreciable effect. – It was therefore decided that, for the main part of the investigation, the digestions should be performed using the domestic sludge itself. This decision became, of course, even more strongly motivated as the experiments became more closely directed towards actual practical conditions.

Formation of Acids

The enrichment of volatile acids in the digestion liquor in some of the trial experiments with cellulose and meat led to the conclusion that these residual acids consisted of such fatty acids which are fermentable only with great difficulty. A determination of the individual acids was therefore made by means of paper chromatography, according to Hiscox and Berridge (7), using concentrates obtained from the steam distillates from three digestions carried out using different proportions between the cellulose and meat. The distillates were neutralized, evaporated down to a small volume, acidified with H_3PO_4 and steam distilled, once again, on a micro scale.

All lower fatty acids from acetic acid up to and including caproic acid were found but the relative amounts were very different in the three cases. In digestion 1 with meat alone as digestion material, it was found that propionic acid and valeric acid were the dominant ones in the mixture. In digestion 2, with 75 % meat and 25 % cellulose, these two acids were not quite so dominant as in digestion 1, and were even less so in digestion 3 in which equal amount of meat and cellulose were used. – Caproic acid was always found in smaller relative amount than the other acids.

The total amount of acids was very high (ca. 4 g/l) in digestion 1, lower (ca. 3 g/l) in digestion 2 and least (ca. 2 g/l) in digestion 3.

To these observations, it should be added that, in the thermophilic fermentation of pure cellulose by means of mixed cultures which lack methane bacteria, the main products are acetic acid, butyric acid and a little formic acid. Therefore, it seems that propionic acid and valeric acid, because they were found in such relatively large amounts in the above mentioned digestion experiments, probably come from the meat. It is of great interest that propionic acid and valeric acid occurred in such large amounts in connection with the unnormally large accumulation of acids in digestion 1. This indicates that these acids are converted more slowly by the methane bacteria than, for example, acetic acid and butyric acid. This conclusion was moreover verified in latter experiments (p. 30). It is also known that, in mesophilic methane fermentation, fatty acids with an odd number of carbon atoms are converted slower than those with an even number. The accumulation of propionic and valeric acids indicates that the same principle with respect to the number of carbon atoms probably also applies in the thermophilic methane fermentation.

(b) 0.75 litre digestions of a mixture of sewage and noll fiber

Industrial celluloses of different types are fermented at greatly different rates by thermophilic cellulose bacteria. In general, it is found that the fermentation rate and fermentability are greatest for that pulp which has been freed, to the greatest possible extent, from lignin and other components of a non-carbohydrate character. For the trial experiments described in (a), a bleached sulphite pulp was used in the form of thin crape paper which is very easily fermentable (for analysis, see p. 4). Digestions on a 0.75 l scale of a mixture of this pulp and fresh sludge showed that the sulphite cellulose is more easily converted than the components in the sludge.

However, in digestions of a mixture of cellulose and fresh sludge under practical conditions, it must be remembered that the cellulose will mainly occur as a waste material, e.g. in the form of noll fiber This material is, relative to the above mentioned bleached sulphite cellulose, fermentable only with difficulty by thermophilic cellulose bacteria. Digestion of a mixture of noll fiber and fresh sludge could therefore be expected a priori to give a different result than what was obtained in the corresponding fermentation with bleached sulphite cellulose.

EXPT. 1. Digestion of a mixture of domestic sewage sludge and noll fiber

0.75 l fermentations were carried out under standardized conditions (p. 6) with the following materials:

Digestion	I:I	I:II	I:III	I:IV
Fresh sludge, g ash-free total solids	0	1.2	2.3	3.5
Noll fiber, g total solids	5.0	4.0	3.0	2.0

The results are shown in Fig. 3.

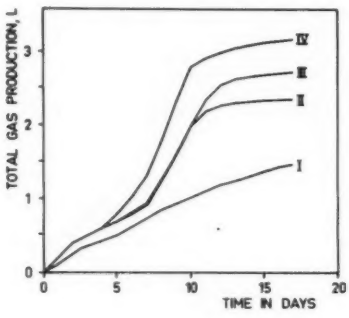

Fig. 3
0.75 l digestion of a mixture of fresh sludge and noll fiber

Noll fiber alone was digested rather slowly but, as soon as fresh sludge was added, the rate increased considerably. Digestion 1:IV progressed with about the same speed as that obtained with sludge without any noll fiber admixture. The situation here was the opposite to that when cellulose (in the form of thin crape paper) was mixed in. The explanation is that the noll fiber contains resinous compounds and other unfermentable substances and can therefore be fermented only with difficulty. This result indicated the desirability of working with those cellulose materials which can occur in practice.

The great disadvantage with digestion experiments on a 0.75 l scale was that each digestion consisted of an isolated step which was started by an inoculation culture in good condition. As later became apparent, it can happen that, in continuous digestions, certain combinations of noll fiber and fresh sludge are digested well for a while but then the process declines due to the fact that the influence of some unfavourable factor has become successively greater.

(c) 8 litre digestions in which the material left undigested was determined during the course of the process

In the digestions reported in this section, the course of the digestion has been followed not only by means of the conventional determinations of material left undigested, volume of gas evolved and amount of volatile acids in the digestion liquor but also by determinations of crude fiber, crude protein and ether soluble in the digestion material during the actual progress of the digestion. In this way, it has been possible to get an idea of the rate at which each component is converted during different phases of the batchwise digestions and, also, whether this conversion rate is constant during the semi-continuous process, thus indicating whether the digestion process becomes stabilized or not.

EXPT. 2. Batch digestion of domestic sewage sludge

8 l diluted fresh sludge was mixed with 650 ml digested sludge as inoculation and, in addition, with nutrient salts etc. according to the standard conditions given on p. 6. The mixture was allowed to digest until tha gas evolution almost ceased.

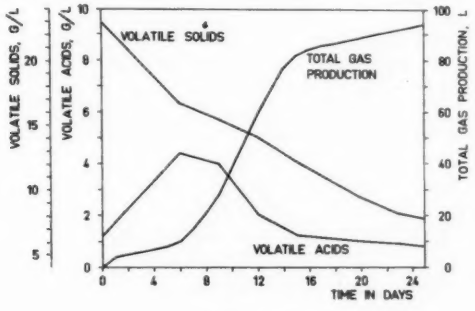

Fig. 4 a

Progress of digestion obtained during batchwise digestion of fresh sludge, Expt. 2.

Volatile solids = filtered solid material as ash-free dry substance
Volatile acids = lower fatty acids in the digestion liquor
"Total gas production" is in this case the amount of gas evolved, not gas volume per l digestion liquor as in the experiments which follow.

Fig. 4 a shows the progress of such a digestion as indicated by the "volatile solids" and the "total gas production". It is seen that the parallelism between digestion material consumed and gas obtained was not quite complete during the experiment because the accumulation of volatile acids in the digestion liquor was so rapid during the earlier part of

the digestion, due to the fact that the methane bacteria could not convert the acids formed sufficiently quickly, that an "acid peak" was obtained. When the methane fermentation had reached its maximum rate, the acid content fell to a normal value.

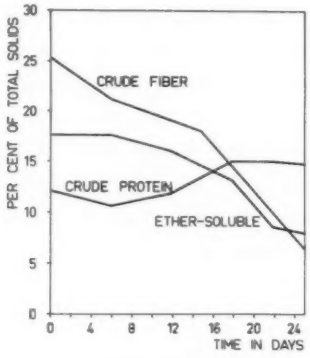

Fig. 4 b

Composition of the as yet undigested material (after filtering off) during batchwise digestion of fresh sludge. Expt. 2.

Crude fiber = cellulose
All values have been expressed as per cent of total dry substance (including ash) i.e. not as per cent of "volatile solids".

In Fig. 4 b, it is seen that the cellulose part of the digestion material is converted very rapidly, thus giving rize to the acid peak just mentioned. The same phenomenon was found in latter experiments.

Table 2

Distribution of components in Expt. 2

	Before digestion g/l	After digestion g/l	Difference g/l	Digested solids %
Total solids	33.0	14.3	18.7	57
Ash	10.1	6.3	-	-
Volatile solids	22.9	8.0	14.9	65
a) Crude fiber	8.4	0.9	7.5	89
b) Crude protein	4.0	2.1	1.9	48
c) Ether soluble	5.8	1.1	4.7	81
Sum of a, b and c	18.2	4.1	14.1	78
Not accounted for	4.7	3.9	0.8	-

The material balance in Table 2 (p. 14) indicates a good decomposition of the cellulose and fat while the protein part had only been converted to ca. 50 % when the digestion was stopped. This slow conversion of the protein had, as stated above, been found already in the first trial experiments.

EXPT. 3. Batch digestion of a mixture of domestic sewage sludge and noll fiber

After a few trial experiments, three parallel digestions with the following media were begun:

Digestion	3:1	3:2	3:3
Fresh sludge, l	6.0	4.5	3.0
Noll fiber, g moist material	-	225	450
Water, l	2.0	3.5	5.0

Each digestion vessel was supplied in addition with a normal portion of nutrient salts and, also, 3.2 g sodium thioglycollate and 700 ml inoculation culture. The latter had been grown on fresh sludge at 58 °C for 3 weeks. The amount of volatile solids and their main components at the start of the digestion are shown in the material balance in Table 3. The course of the digestion and the composition of the material still left undigested is shown in Fig. 5 (a) – 5 (f), (p. 16).

The course of 3:1 (which was performed with fresh sludge of a different date to that for Expt. 2, p. 13) was analogous to that for Expt. 2. Part of the cellulose was converted rapidly during the first phase of the digestion, thus resulting simultaneously in an accumulation of the volatile acids. In 3:1, the amount of cellulose was not so large that the acid peak had any serious effect on the gas production.

Upon admixture of noll fiber (3:2 and 3:3), the difference between the rates for the cellulose decomposition and gas evolution was accentuated due to the fact that the gas evolution was depressed to a certain extent by the rapid accumulation of the volatile acids.

Even though the solid digestion material was converted at about the same rate in all cases, the gas evolution was depressed in 3:2 and in particular in 3:3. The total amount of gas evolved in these last two digestions would therefore have been larger if the digestion time had been increased by a few days.

The tendency towards the formation of acid peaks is a disadvantage in the batchwise experiments because the accumulation of volatile

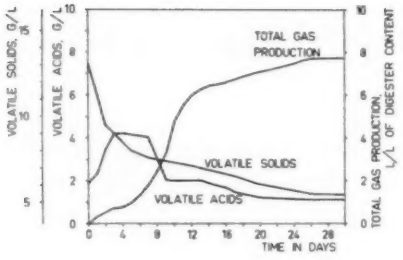

Fig. 5 a

Progress of digestion in Expt. 3:1
Digestion material consisted solely of fresh sludge

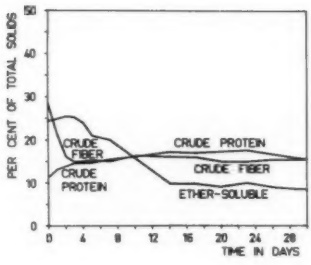

Fig. 5 b

Composition of the as yet undigested material in Expt. 3:1

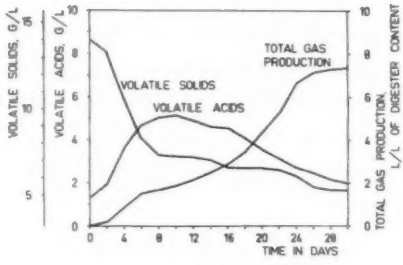

Fig. 5 c

Progress of digestion in Expt. 3:2
Digestion material: fresh sludge and noll fiber in the ratio 2:1

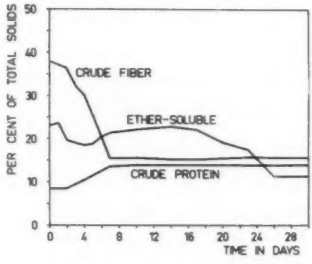

Fig. 5 d

Composition of the as yet undigested material in Expt. 3:2

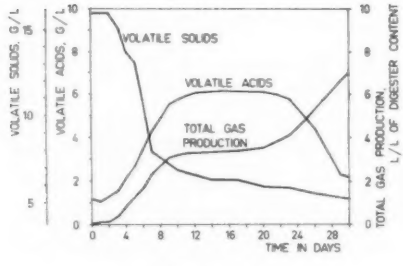

Fig. 5 e

Progress of digestion in Expt. 3:3
Digestion material: fresh sludge and noll fiber in the ratio 1:1

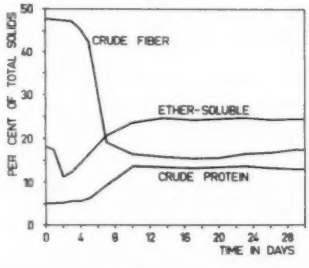

Fig. 5 f

Composition of the as yet undigested material in Expt. 3:3

Table 3

Distribution of components in Expt. 3

	1				2				3			
	Before digestion g/l	After digestion g/l	Difference g/l	Digested solids %	Before digestion g/l	After digestion g/l	Difference g/l	Digested solids %	Before digestion g/l	After digestion g/l	Difference g/l	Digested solids %
Total solids	18.5	10.6	7.9	43	19.6	9.6	10.0	51	20.8	8.7	12.1	58
Ash	6.2	4.9	1.3	-	5.6	4.3	1.3	-	4.9	3.5	1.4	-
Volatile solids	12.3	5.7	6.6	54	14.0	5.3	8.7	62	15.9	5.2	10.7	67
a) Crude fiber	5.2	1.7	3.5	67	7.4	1.5	5.9	80	9.9	1.5	8.4	85
b) Crude protein	2.1	1.7	0.4	19	1.6	1.4	0.2	13	1.1	1.1	0.0	0
c) Ether soluble	4.5	0.9	3.6	80	4.5	1.1	3.4	76	3.8	2.1	1.7	45
Sum of a, b and c	11.8	4.3	7.5	64	13.5	4.0	9.5	70	14.8	4.7	10.1	68
Not accounted for	0.5	1.4	-	-	0.5	1.3	-	-	1.1	0.5	-	-

acids, as well as depressing the gas evolution, can also have an injurious effect on other bacterial processes. The influence of acid peaks can to a certain extent be counteracted by using more dilute suspensions but a better way is to use a semi-continuous digestion as described in Expt. 4.

Table 3 shows the decomposition in g/l and also in per cent of the solid digestion material and its main components. (Sporadically occurring irregularities in the values are probably mainly due to unavoidable sampling errors.)

Admixture of noll fiber caused the following important changes:

(a) Better percentage decomposition of the total organic matter.

(b) Poorer decomposition of the fat and protein (in 3:3 no protein decomposition at all could be detected).

EXPT. 4. Semi-continuous digestion of a mixture of domestic sewage sludge and noll fiber without addition of ammonia-N.

Semi-continuous digestions were performed by removing regularly (each or every second day) a definite amount of digestion liquor and replacing it by an equal volume of water and fresh digestion material. Three parallel digestions with the following additions were performed:

Digestion	4:1	4:2	4:3
Fresh sludge, %	100	50	25
Noll fiber, %	0	50	75

The amount of fresh sludge is expressed with reference to ash-free total solids while the amount of noll fiber is expressed with reference to resin- and ash-free total solids. No nutrient salts were added. The series was started by adding 0.5 l/day of the above mentioned additions to 8 l digesting fresh sludge in each apparatus until stable conditions had been reached after ca. 1 month. Thereafter, the additions were made according to the line marked "feed rate" in Fig. 6 (a) - 6 (f). These diagrams show the subsequent course of the digestion (after 1/12 1958).

The gas evolution in digestion 4:3 stopped completely after ca. 25 days and that in 4:2 after ca. 35 days. In both cases, the content of volatile acids increased considerably towards the end of the experiment and this was probably the main reason why the methane fermentation stopped. In both 4:2 and 4:3, it appears that the amount of ammonia-N added by means of fresh sludge was insufficient since the content of ammonia-N in the digestion liquor decreased successively to zero. Digestion 4:3 presented a pathological picture right from the beginning with its con-

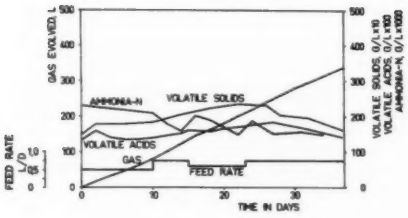

Fig. 6 a

Progress of digestion in Expt. 4:1
Digestion material consisted solely of fresh sludge

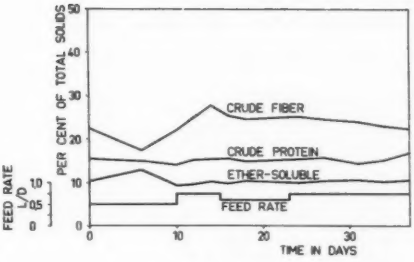

Fig. 6 b

Composition of the as yet undigested material in Expt. 4:1

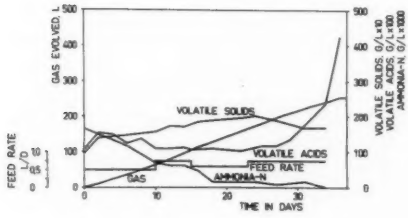

Fig. 6 c

Progress of digestion in Expt. 4:2
Digestion material: fresh sludge and noll fiber in the ratio 1:1

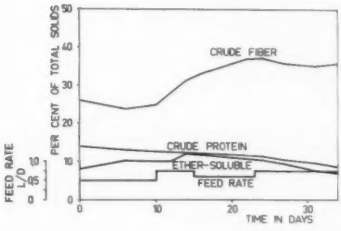

Fig. 6 d

Composition of the as yet undigested material in Expt. 4:2

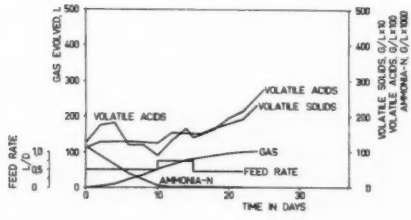

Fig. 6 e

Progress of digestion in Expt. 4:3
Digestion material: fresh sludge and noll fiber in the ratio 1:3

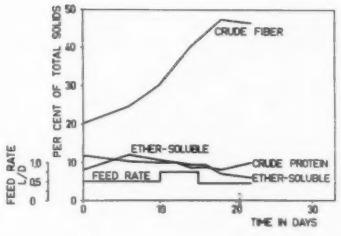

Fig. 6 f

Composition of the as yet undigested material in Expt. 4:3

tinuously increasing percent of crude fiber in the solids still left undigested. The gas evolution in 4:2 was much lower than that in 4:1 while that in 4:3 was almost negligible. Summarizing, it can be said that conditions such as those in 4:2 and 4:3 do not seem to be applicable for a semi-continuous process.

EXPT. 5. Semi-continuous digestion of a mixture of domestic sewage sludge and noll fiber with the addition of ammonia-N.

In the laboratory cellulose fermentations and methane fermentations empirical media to which ammonia-N has been added (corresponding to 1-2 g $(NH_4)_2SO_4$ per litre) are used. A new series of digestions (viz.: 5:1 – 5:3) was started in the same way and under the same conditions as in Expt. 4 but with the difference that the content of ammonia-N in the digestions with a noll fiber addition was maintained at its initial value by means of regular additions of NH_4Cl.

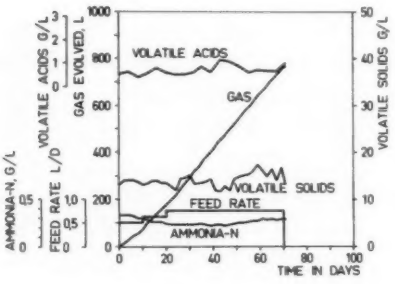

Fig. 7 a

Progress of digestion in Expt. 5:1
Digestion material consisted solely of fresh sludge

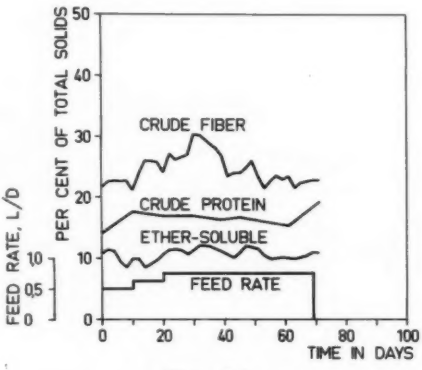

Fig. 7 b

Composition of the as yet undigested material in Expt. 5:1

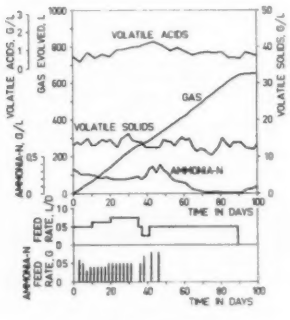

Fig. 7 c

Progress of digestion in Expt. 5:2
Digestion material: fresh sludge and noll fiber in the ratio 1:1

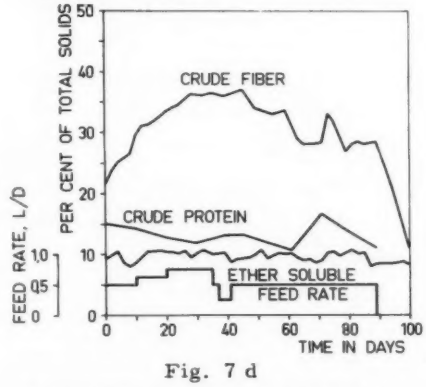

Fig. 7 d

Composition of the as yet undigested material in Expt. 5:2

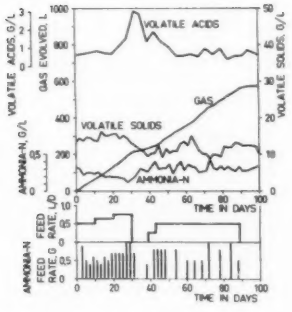

Fig. 7 e

Progress of digestion in Expt. 5:3
Digestion material: fresh sludge and noll fiber in the ratio 1:3

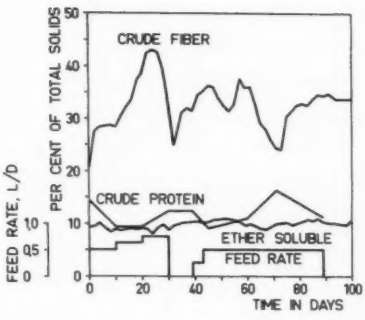

Fig. 7 f

Composition of the as yet undigested material in Expt. 5:3

During the first ten days, the progress of the fermentation was normal (see figs. 7 (a) – 7 (f)). The addition of fresh digestion material, which occurred every second day, consisted during this period of 0.5 l/day. Due to the desirability of coming up to as high a conversion as possible, these additions were first increased to 0.6 and then to 0.75 l/day. This resulted in, however, an overloading in 5:2 and 5:3. In 5:3, a considerable acid accumulation occurred but this could be eliminated by not making any more additions of digestion material for the next 8 days. The situation was analogous in 5:2, although not to such a pronounced degree, and even in this case it was necessary to decrease, during 4 days, the addition of fresh digestion material to half. After the processes in 5:2 and 5:3 had become normal once again, it was possible to keep the digestions going using an addition of 0.5 l/day. It is clear that the maximum capacity lies somewhere between 0.5 and 0.75 l/day. Digestion 5:1 with fresh sludge alone could be kept going without any trouble using an addition of 0.75 l/day. No investigation was performed to determine the maximum capacity since we did not wish to risk overloading.

After it had become obvious that stable conditions had been satisfactorily established, the addition of NH_4Cl to 5:2 was stopped in order to produce, due to the considerable decrease in the nitrogen supply, a shock effect similar to that in the digestions 4:2 and 4:3. Since the conditions in 5:2 were more favourable than those in 5:3, in which the amount of noll fiber was largest, it would seem that such a shock effect in this case would further emphasize the influence of a lack of nitrogen.

Table 4

Distribution of components in Expt. 5

	1				2				3			
	Day 40 - 60				Day 50 - 85				Day 50 - 85			
	Before diges- g/l	After diges- g/l (mean value)	Diffe- rence g/l	Digested solids %	Before diges- g/l	After diges- g/l (mean value)	Diffe- rence g/l	Digested solids %	Before diges- g/l	After diges- g/l (mean value)	Diffe- rence g/l	Digested solids %
Total solids	36.5	20.0	16.5	45	36.2	19.1	17.1	47	36.0	16.9	19.1	53
Ash	6.5	5.9	-	-	6.2	6.1	-	-	6.0	5.6	-	-
Volatile solids	30.0	14.1	15.9	53	30.0	13.0	17.0	57	30.0	11.3	18.7	62
a) Crude fiber	10.9	4.7	6.2	57	16.2	5.8	10.4	64	18.6	5.3	13.3	72
b) Crude protein	5.6	3.2	2.4	43	2.9	2.6	0.3	10	1.5	2.3	- 0.8	0
c) Ether soluble	5.9	2.2	3.7	63	3.9	1.9	2.0	51	3.1	1.7	1.4	45
Sum of a, b and c	22.4	10.1	12.3	55	23.0	10.3	12.7	55	23.2	9.3	13.9	60
Not accounted for	7.6	4.0	-	-	7.0	2.7	-	-	6.8	2.0	-	-

It was found, however, that the 5:2 digestion continued normally despite the fact that the content of ammonia-N in the digestion liquor ultimately decreased to zero. When, eventually, the series had to be stopped due to lack of time, there was still no sign of 5:2 going wrong. This result is very positive from a practical point of view but it indicates that the question concerning the necessity of an ammonia-N addition has not been subject to sufficient experimental investigation. The experiment shows, however, that it is possible to semi-continuously digest a mixture of 1 part fresh sludge and 3 parts noll fiber with a daily addition of $1/16^{th}$ (6.25 %) of the digestion liquor volume if the liquor contains 0.3 – 0.4 g/l ammonia-N. The maximum addition is however still rather uncertain and, moreover, we have not had time to determine how much ammonia-N must be added. It is however probable that the maximum addition of digestion material requires, unavoidably, a certain definite addition of ammonia-N.

The material balance for digestions 5:1 – 5:3 is shown in Table 4. It is striking that the same tendency as in the batchwise digestions occurs in this case. An increase in the noll fiber resulted in a good digestion of the cellulose part of the digestion material but also in a poorer degree of digestion of the fat and, in particular, the protein as in the case of fresh sludge alone.

It seems as though a sort of competition or, possibly, antagonism exists between the cellulose-decomposing bacteria and the protein-decomposing ones. In practice, it ought to be possible to counteract this effect by performing the digestion at a somewhat lower temperature than that used here. The conversion of the noll fiber would, however, then be less rapid than in the present case.

The composition of the digestion gas was calculated under the assumptions given in Table 5.

Table 5

Theoretical results for digestion of carbohydrates, proteins and fats according to Roediger (8)

Material	Quantity of gas, ml/g	Composition of gas CH_4, %	CO_2, %
Carbohydrate	790	50	50
Protein	704	71	29
Fat	1250	68	32

Using these figures as a basis for the calculations, the following "theoretical" values were obtained for the converted material in Table 4, ('Fat' = 'Ether soluble').

Table 6

Theoretical values for gas volume and composition in expts. 5:1 - 5:3

Material	Quantity of gas ml/g converted digestion material	Composition of gas CH_4, %	CO_2, %
5:1	910	60.6	39.4
5:2	860	54.6	45.4
5:3	830	52.6	47.4

These values differ however from the experimental ones due to the fact that a certain quantity of CO_2 was bound in the weakly alkaline digestion liquor in the beginning but was, then, partially lost when the digestion liquor was successively replaced with new digestion material. Thus, we found on two occasions:

Table 7

Experimental values for the composition of gas in expts. 5:1 - 5:3

Digestion	Composition of gas CH_4, %	CO_2, %
5:1	64 63	36 37
5:2	61 59	39 41
5:3	60 54	40 46

Therefore, a determination of the ratio $CH_4 : CO_2$ in the digestion gas produced by a semi-continuous digestion seems to be worthless as far as an accurate check on the result of the digestion is concerned. The pH and buffering capacity of the digestion liquor vary so much that the quantity of CO_2 which is bound in the liquor cannot be determined with sufficient accuracy.

2. Bacterial Investigations

In the digestion of a mixture of cellulose and protein, at least three different types of bacteria must co-operate. The cellulose and protein are subjected to hydrolysis, brought about by at least two different types of bacteria (cellulose bacteria and protein bacteria), and, thereafter, the final methane fermentation is performed by special methane bacteria. In practice, the digestion material contains more components than these and the necessary bacterial flora will therefore be much more complicated. In addition, there can exist, between the initiating hydrolysis of the solid substances and the final methane fermentation, a number of intermediate substances which in their turn are either produced or converted by further types of bacteria. From a bacteriological point of view, sludge digestion seems to give rise to a very variegated collection of bacteria and bacterial reactions. The investigation of such a complicated system would certainly be very difficult and tedious. This supposition is corroborated by the fact that the amount of literature published in this field is very small.

We have not been able to find any information in the literature concerning thermophilic, protein-decomposing bacteria. Thermophilic cellulose fermenters have been isolated by a few research workers, amongst others by one of us (16). The isolation is hazardous. An isolation of methane bacteria was attempted by Barker and coworkers and was found to be both troublesome and also often resultless from the point of view that the pure cultures obtained could not be sub-cultured further. Summarizing, it can be said that it is much more difficult to isolate cellulose fermenters and, in particular, methane bacteria than most of the other bacteria of technical importance.

However desirable it would have been to have access to and to study pure cultures of as many of the bacteria acting during sludge digestion as possible, it is clear that a semi-technical investigation of the present type cannot be directed towards such problems to any great extent. The information collected under "Bacterial Investigations" does not result from any detailed investigations but rather from single experiments and observations.

(a) Isolation in pure culture of a thermophilic protein-digesting bacterium

A suspension of minced meat in water was inoculated with digested sludge and then incubated anaerobically at 55°C. The meat fermented rapidly. After sub-culturing in the same medium a number of times, the

culture was purified by plating on Difco nutrient agar containing Na-thioglycollate. After further cultivation in the meat medium in the presence of nutrient salts and thioglycollate, it was possible to obtain, after plating according to Gibbs & Hirsch (9) a pure culture with the characteristics shown below.

The isolation was easy and involved none of the complications which accompany the isolation of cellulose fermenters and methane bacteria.

Properties:

Strictly anaerobic. Good growth at 55°C and somewhat slower at 37°C no growth at all at 63° or 25°C. The cells consist of straight, occasionally somewhat bent, mobile gram negative rods with round ends, 0.5 x 3 - 5 μ. Terminal spore formation accompanied by a swelling of the spore-bearing cell.

On agar according to Gibbs and Hirsch: very sparse growth on the surface. Young (18 h) surface colonies: ca. 1 mm in diameter, round, slightly raised, smooth, shiny, greyish white, opaque. Older (14 days) surface colonies: somewhat uneven edge and rough surface. Colonies odourless.

Deep colonies small, lens-shaped.

Catalase negative. Liquifies gelatine, decomposes meat particles which are first coloured red. Does not hydrolyze cellulose and does not grow in starch-agar.

(b) On the reaction mechanism of thermophilic methane fermentation

It seems most likely that the formation of methane in sludge digestion always occurs in fundamentally the same way independent of whether it is performed using mesophilic or thermophilic methane bacteria. Information concerning the actual mechanism in thermophilic methane fermentation seems to be lacking in the literature. A certain amount of support for the proposition that mesophilic and thermophilic methane fermentations are similar processes is provided by Buswell's summarizing formula which appears to apply independent of the temperature (10).

$$(1)\quad C_nH_aO_b + (n - a/4 - b/2)\,H_2O = (n/2 - a/8 + b/4)\,CO_2 + (n/2 + a/8 - b/4)\,CH_4$$

which implies that the compound $C_nH_aO_b$ is completely converted into methane and carbon dioxide. This formula has been found to apply for

carbohydrates, proteins and fats in both the mesophilic and thermophilic regions.

Buswell's formula includes two separate processes, viz. partly the breaking-down of the raw material $C_nH_aO_b$ to lower fatty acids and aliphatic alcohols, and partly the conversion of the latter to methane and carbon dioxide. The fact that Buswell's formula applies, in addition, for the thermophilic region means that the primary breaking-down to fatty acids and alcohols occurs quantitatively also in this case so that no accumulation of intermediate products takes place. Such a disturbance in the process would soon be noticed in a purification plant. On the other hand, the fact that Buswell's formula applies does not give any answer to the question about how the thermophilic formation of methane takes place in detail. We shall return to this question later in connection with the discussion of certain experiments which will be described in the following section. A general survey of the reaction mechanism of the methane fermentation is made difficult by the fact that the methane is formed in at least two different ways. Thus, Barker (11) found that the production of methane from alcohols occurs via a reduction of CO_2, which is formed as an intermediate product, to CH_4. Later, Stadtman and Barker (12) showed that, in the methane fermentation of acetic acid, the methane originates from the methyl group.

As a starting point for the experiments on the ability of the thermophilic methane bacteria to decompose different compounds, we have taken the following reactions of which the fatty acid decomposition, according to Lynen, under the influence of coenzyme A does not seem to have been paid attention to in this connection.

(1) Fatty acid decomposition according to Lynen

This reaction implies that 1 mol acetyl-CoA is split off via a cyclic process from higher fatty acids, i. e. the carbon skeleton of such an acid loses two carbon atoms for each turn in the process. If the original fatty acid has an even number of carbon atoms, this will lead to a complete decomposition into acetic acid (as acetyl-CoA) while, if the number is odd, then the cycle will be broken off at, for example, the propionic acid stage. This hypothesis would explain why fatty acids with an odd number of carbon atoms are converted so sluggishly. This applies to valeric acid, and, in particular, to propionic acid.

The acetic acid is then converted into CH_4 and CO_2 by means of a direct decarboxylation:

$$3\ CH_3COOH \longleftarrow 3\ CH_4 + 3\ CO_2$$

(2) Oxidation-reduction reaction between an aliphatic alcohol and CO_2 giving methane.

This reaction has been observed with ethanol and butanol (11, 13). For example:

$$2\ C_2H_5OH + CO_2 \longrightarrow 2\ CH_3COOH + CH_4$$

The fatty acid formed is then converted via (1) above.

As mentioned on p. 30, we found an accumulation of fatty acids, especially propionic acid, when fermenting ethanol and methanol. It is conceivable, at least in the case of ethanol, that an oxidation-reduction reaction takes place with a subsequent synthesis by means of Lynen's cycle.

(3) Direct reduction of CO_2 to CH_4 with DPNH*).

(4) Reduction of a fatty acid with DPNH to the corresponding alcohol, followed by an oxidation-reduction reaction between this alcohol and CO_2.

Elective cultures of methane bacteria

Sludge-digesting mixed cultures, containing thermophilic methane bacteria, are easy to obtain but experiments with them do not give any information concerning the ability of the methane bacteria to convert a certain substance provided that it is not of such a type that it can only be utilized by the methane bacteria. The experimental conditions existing in sludge digestion imply that one can a priori assume that, for example, methyl and ethyl alcohol and acetic acid are not converted, in such cases, by other bacteria than methane bacteria. On the other hand, the extensive investigations on fermentations with mixed cultures of a large number of chemically pure compounds, which were performed during the 1930s by Buswell and coworkers (14), give little information about how the methane bacteria in themselves react towards the substances tested.

As has been pointed out above, experiments with pure cultures of methane bacteria come up against great difficulties as far as the preparation and maintenance of the cultures at full activity are concerned. Because the methane bacteria are so specialized, it is however possible to obtain results of value using elective cultures, provided that these results are judged carefully.

Elective cultures are prepared by natural selection, i.e. the culture is grown under such conditions that the desired bacterial type domi-

*) DPNH = reduced diphosphopyridine nucleotide

nates completely. Using this method, it is often possible to prepare cultures which appear microscopically and physiologically to be absolutely pure cultures but it has to be remembered that a small amount of foreign bacteria will always be present. If the experimental conditions are altered so that these foreign bacteria become favoured, then the elective culture will soon return a more undefined mixed culture type. Such a reversion requires a certain time and it is on this circumstance that the experiments, described below, are based.

Ever since Barker in 1936 described a suitable technique for use with elective cultures, most of the investigations on the mechanism of methane fermentation have been performed using such cultures.

As starting point for our experiments, we chose elective cultures which had been developed by means of successive additions of small amounts of acetic acid as energy source (concerning the technique for this, see below). When the last addition of acetic acid had been almost completely fermented, carbon sources of different types were added and the behaviour of the culture was judged by means of the gas evolution in the following way:

(α) The gas evolution continued, without any intermediate stoppage, after the last trace of acetic acid had been used up, i.e. the carbon source was converted directly by the methane bacteria.

(β) Quickly ceasing gas evolution, i.e. the carbon source was not converted by the methane bacteria.

(γ) Same result as in (β) but, after a time, the gas evolution started up again. This can mean that the methane bacteria needed a certain time for acclimatization to the added substance or that the subsidiary flora eventually developed and became effective for the conversion or that the methane bacteria and the subsidiary flora together attacked the substance in question. A fermentation course accordning to (γ) therefore provide little information and requires a complementary investigation.

Elective cultures were prepared in the following way:

From sludge cultures, adapted to 58 - 60°C, 75 ml was inoculated into 675 ml nutrient solution in 1 l bottles (cf. page 6). Acetic acid, neutralized with NaOH to pH 7.5, was added as energy source. When the fermentation had started, a suitable quantity of acid was added at equal intervals (in general every second day) so that a stable fermentation process with a maximum fermentation rate was obtained. Addition of NaOH was not found to be necessary but the pH was checked daily and

adjusted when necessary to 7.5. After a certain time, 75 ml of the contents in the bottles was inoculated into new bottles with the same medium and the whole procedure repeated a certain number of times. In this way, stable, elective cultures were eventually obtained which had been adapted to acetic acid. In the experiments with different carbon sources, which will be described in the following, the carbon source in question was added instead of acetic acid and the amounts added were chosen as before so that a maximum conversion rate was maintained.

The bottles and medium were sterilized and the additions and pH adjustments were performed aseptically. This was done in order to exclude other organisms than those which had been introduced into the cultures by means of the inoculations.

EXPT. 6. Fermentation of different carbon sources by elective methane-producing cultures.

(a) Methanol, ethanol, lower fatty acids, ethyl acetate

The results of fermentations made with these compounds are shown in Figs. 8 and 9.

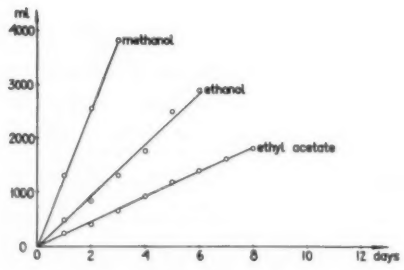

Fig. 8

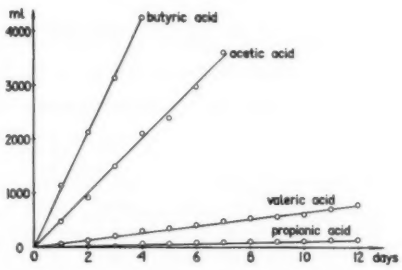

Fig. 9

Fermentation of different carbon sources by elective methane-producing cultures

Methanol was a more favourable substrate than ethanol. Fatty acids with an even number of carbon atoms were fermented much more easily than those with an odd number. The fermentation rates for butyric and propionic acids were in the ratio of about 100:1. Propionic acid clearly constituted a sort of final stage while it was still possible to break down valeric acid. The lack in ability of the bacteria to ferment propionic acid at an appreciable rate explains why this acid became accumulated in the trial fermentation on a 8 l scale (page 9).

Fig. 8 also contains a curve for ethyl acetate. The conversion mechanism for this compound is at present unclear because a non-enzymatic hydrolysis is also possible.

When the fermentation of ethanol and methanol was allowed to continue for a long time, a certain accumulation of lower fatty acids, in particular propionic acid, was observed. Probably, the alcohols were converted by means of some unknown mechanism to acetic acid which then (via Lynen's fatty acid cycle?) gave propionic acid and traces of higher acids. This fatty acid synthesis indicates that it is not possible simply to assume that culture on methanol and ethanol will result in a selection amongst the methane bacteria so that the fatty acid-fermenting types will be completely eliminated in favour of only alcohol-fermenting ones (to the extent that such exist).

(b) Higher fatty acids

Cultures, adapted to butyric acid, were provided successively with caproic, caprylic, capric, lauric and myristic acids. The change over to a higher acid occurred only after the fermentation with the proceeding one had become stabilized. All of the higher acids were added as emulsions in hot water. The results are shown in Fig. 10.

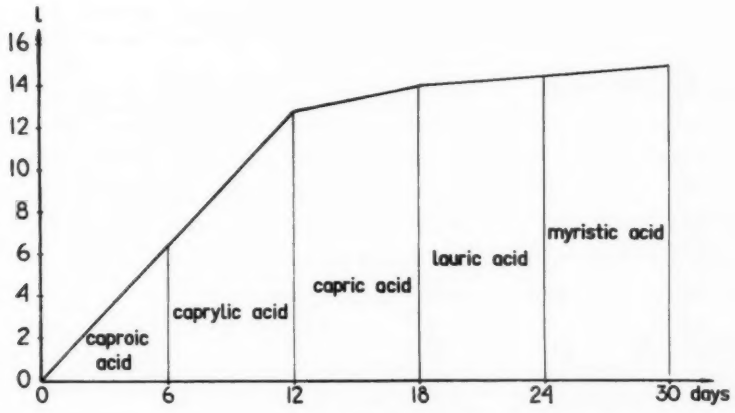

Fig. 10

Fermentation of higher fatty acids by elective methane-producing cultures

From (and including) capric acid onwards, the fermentation proceeded relatively slowly. It may be questioned whether this is related to the decreasing solubility in water of the higher fatty acids when the number of carbon atoms is increasing.

(c) Glycin

Because it seemed possible that methane bacteria could deaminate at least the simplest types of amino acids, glycin was added to a culture grown on acetic acid. The fermentation however quickly stopped, thus indicating that the methane bacteria cannot utilize glycin. No deaminating subsidiary flora appeared.

(d) Acids in Krebs' cycle

A characteristic property of methane bacteria has been considered to be that they are adapted almost exclusively to alcohols and fatty acids as an energy source. This specialization with respect to the substrate is without doubt very characteristic but it may nevertheless be questioned whether it is complete. We decided therefore to investigate whether the elective cultures could also convert the acids in Krebs' citric acid cycle even if two important reservations can be made against such a choice:

(α) The acids in the citric acid cycle are often very poorly converted by intact cells (permeability difficulties).

(β) The cycle is typical for aerobic and facultative anaerobic bacteria while it has not been reported to be performed by strictly anaerobic bacteria.

On the other hand, the acids occurring in the citric acid cycle are so important as initial and intermediate products in different biochemical connections that they should also be important for methane bacteria, even if the latter do not use the cycle as an energy producing process. Trial experiments using additions of these acids showed in fact that the methane bacteria were able to attack them to a certain extent and, thus, further tests were performed in the following way:

Each of the acids in the citric acid cycle (oxalosuccinic acid not tested) were added to elective cultures which had been grown on acetic acid. In addition pyruvic acid was investigated. The amounts of the additions were such that 1 l of gas should have been evolved at complete conversion. The fermentations were allowed to proceed until the gas evolution ceased. Table 8 contains the mean values for two series.

Table 8

Amount of gas evolved in the fermentation of the acids in the citric acid cycle (theoretical amount of gas: 1000 ml).

Acid	Ml gas
Oxaloacetic acid	470
Malic acid	600
Fumaric acid	300
Succinic acid	40
α-ketoglutaric acid	300
Isocitric acid	500
Cis-aconitic acid	450
Citric acid	670
- - - - - -	
Pyruvic acid	570

IV. SUMMARY AND DISCUSSION

The main purpose of this investigation has been to examine certain basic problems which can arise in the thermophilic digestion of a mixture of domestic sludge from a sewage purification plant and cellulose sludge from a pulp factory.

Trial experiments, both batchwise and semi-continuous, showed that the balance between carbohydrate and nitrogen nutrition, which should have been obtained when digesting a mixture of cellulose and protein materials, did not occur due to the fact that these materials were converted at greatly different rates. In these experiments, cellulose in the form of bleached sulphite pulp and protein in the form of meat, casein or (best of all) egg albumin were used. The cellulose was digested much quicker than these latter materials. In addition, these proteins gave rise to a conderable accumulation of lower fatty acids with an odd number of carbon atoms (especially propionic and valeric acids) in the digestion liquor. Since these acids are converted very slowly by the methane bacteria, it is clear that even a moderate addition of protein to the digestion material will greatly increase the risk that the content of lower fatty acids in the digestion liquor will become so great that the formation of methane will be suppressed.

Because it seemed that the above mentioned phenomenon was caused by the fact that the digestion occurred in a medium of much more artificial character than domestic sludge, additions of B group vitamins were made in the continuous digestions but were without result. The experimental conditions were therefore altered, the digestions being performed with autoclaved domestic sludge mixed with sulphite noll fiber. It is true that a more complicated and less reproducible digestion material was obtained in this way but, on the other hand, the combination described above corresponds rather well to the material occurring under actual practical conditions. Sulphite fiber is in itself only digested with great difficulty by thermophilic cellulose bacteria while fresh domestic sludge without admixture is easily digested under the conditions employed here.

The course of the various digestion experiments was followed by means of analyses on the material left undigested. This permitted an estimation of the individual rates with which the cellulose, protein and fat were converted. In addition, routine determinations were made on the gas evolution and content of lower fatty acids in the digestion liquor.

In trial digestions on a 0.75 l scale, noll fiber was converted easier when it was mixed with fresh domestic sludge. Even a small addition of fresh sludge had a favourable effect.

In the batchwise digestions on a 8 l scale of mixtures of fresh sludge and noll fiber, in which the fresh sludge was the dominant component, it was found that the cellulose content of the digestion material was converted more quickly than the fat and protein. The breakdown of the cellulose took place so rapidly that it caused a temporary accumulation of volatile acids with a resulting depression of the gas production. The material balance indicated that percentage break down of the fat and, in particular, the protein was smaller when the amount of noll fiber in the mixture was increased. The total decomposition of digestion material was greater with the maximum amount of noll fiber than with fresh sludge alone.

Corresponding semi-continuous digestions on an 8 l scale of a mixture of fresh sludge and noll fiber showed that an increase in the proportion of noll fiber resulted in a decrease in the digestion rate. The conversions of cellulose, protein and fat were in themselves analogous to those in the batchwise experiments. When the amount of noll fiber was increased, the break down of the cellulose increased while that of the fat and, in particular, the protein decreased. With 75 % noll fiber (ash- and

resin-free substance) and 25 % fresh sludge (ash-free substance), no decomposition of the protein was found.

With 50 % and 75 % noll fiber, the content of ammonia-N in the digestion liquor sank to zero, probably because the protein decomposition was negligible and because an amount of ammonia-N corresponding to a content of 0.3 – 0.4 g/l seemed to be necessary for the maintenance of the digestion process.

It seems probable that a mixture of 75 % noll fiber and 25 % fresh sludge can be satisfactorily digested using a semi-continuous process according to the above, provided that the process is kept under good analytical control, that a somewhat lower digestion temperature is used than in our experiments in order to stimulate the protein decomposition and that, if the content of ammonia-N tends to fall below 0.2 g/l, nitrogen is added in the form of an ammonium salt.

Parallel with the more technical digestion experiments, several investigations were performed, using elective cultures, on the physiology of the thermophilic methane bacteria. Thus, it was investigated to what extent these bacteria could utilize certain carbon sources of basic importance. Methanol and ethanol were fermented very easily while glycin could not be used as an energy source. A certain accumulation of lower fatty acids was observed in the fermentation of methanol and ethanol. Pyruvic acid and most of the acids occurring in the citric acid cycle were fermented, although not completely.

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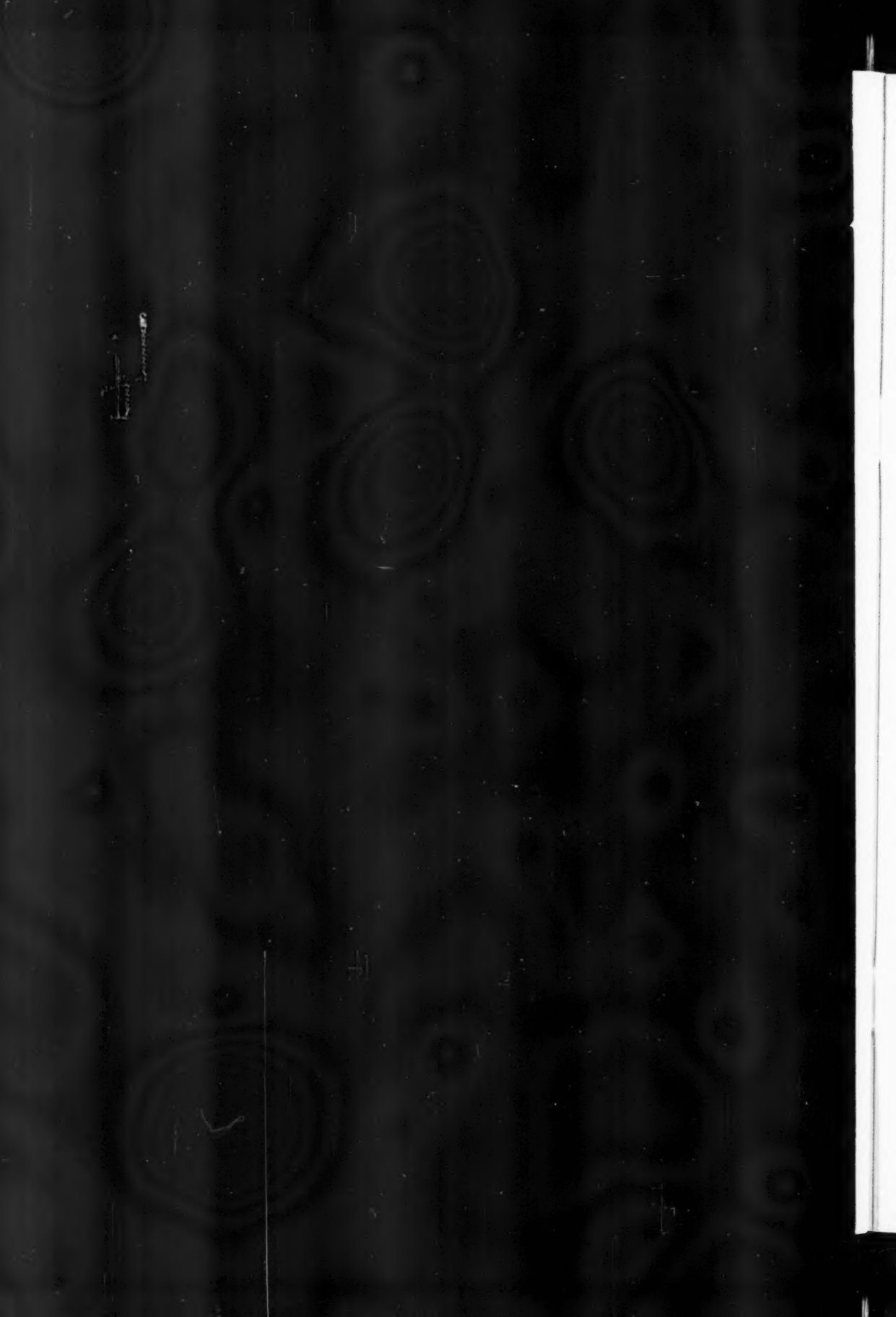

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